



Evaluation of an Antibacterial Effect of Hibiscus Rosa Sinensis Leaves and Petals Extract Along with Antibiotics on *Escherichia coli* : In vitro study

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ABSTRACT

Background: Nature has been a source of medicinal agents for thousands of years and a striking number of modern drugs have been isolated from natural sources, many based on their use in traditional medicines or phytomedicines. As the plants have co-evolved with pathogens, they have also developed the chemical protection pathways against the organisms. Therefore, varieties of plants compounds are associated with antimicrobial and antibacterial activities. The hibiscus rosa sinensis plant extract have multiple organic component like flavonoids, tannins, alkaloids, triterpenoids which are known to have antibacterial activity against *E. coli*, *P. aeruginosa*, *Salmonella species*. Phytotherapy is considered to be less toxic and minimal or no side effects in comparison to modern allopathic medicines. Therefore, in today's scenario there has been reappearance of interest developed in herbal medicine. Therefore, the study was planned to look for antibacterial activity of extract of petals and leaves of Hibiscus rosa sinensis against

Material and Methods: *Escherichia coli* isolated from different clinical samples along with resistant antibiotics. All resistant isolates were tested along with Hibiscus Rosa Sinensis extract both leaves and petals extract with resistant antibiotics.

Results: We found that among 35 (68.63%) resistant *E. coli* isolates to different antibiotics and 85.72% were from urine samples. Enhancement effect in zone size with many resistant antibiotics was seen individually with both extracts. Both extracts showed equal enhancement in zone size of cefotaxime (20.83%) and amikacin (25%). No effect of extracts was seen on levofloxacin.

Key Words: Hibiscus Rosa Sinensis, petal extract, leaves extract

INTRODUCTION

Environment of hospitals serves as a reservoir for various microorganisms; which may be resistant to multiple antibiotics and the selective pressure of antimicrobial used in hospitals, therefore makes an environment a repository for these resistant strains [1,2,3]. However, selective pressure exerted by antimicrobials drug use also has been the major driving force behind the emergence and spread of drug resistant traits among pathogenic and commensal bacteria.

Surveillance data showed that resistance in *E. coli* is consistently highest for antimicrobial agents that have been in

use for the longer time in human and veterinary medicine [4]. The emergence of *E. coli* isolates with multiple-antibiotic resistance phenotypes, involving co-resistance to four or more unrelated families of antibiotics has been reported and is considered a serious health concern [5].

Antimicrobial drug resistance (ADR) hampers the control of infectious diseases and has potential to threaten health security, damage trade and economies but it is difficult to think of "the world without antibiotics". Most of the pathogenic bacteria have developed resistance to modern antibiotics also.

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We know that, nature has been a source of medicinal agents for thousands of years. As the plants have coevolved with pathogens, they have also developed the chemical protection pathways against the organisms. Therefore, varieties of plants compounds are associated with antibacterial activities.

The Hibiscus Rosa Sinensis plant extract have multiple organic component like flavonoids, tannins, alkaloids, triterpenoids which are known to have antibacterial activity against *E. coli*, *P. aeruginosa*, *Salmonella species* [6]. Phytotherapy is considered to be less toxic and minimal or no side effects in comparison to modern medicines. Therefore, in today's scenario there has been reappearance of interest developed in herbal medicine.

So, the study was planned to evaluate the antibacterial effect of petals and leaves extract of Hibiscus Rosa Sinensis along with resistant antibiotic against *Escherichia coli* isolated from different clinical samples.

MATERIALS AND METHOD

This is a hospital based prospective study from April to September 2019. We have included 51 *Escherichia coli* isolated from different samples during the study period. *E.coli* were isolated and identified as per routine standard protocol. All *E. coli* isolates were first tested for routine antibiotic by Kirby Bauer disc diffusion method as per CLSI guideline [7]. Then, isolates which was found to be resistant to one or more antibiotics was further tested by using Hibiscus Rosa Sinensis petals and leaves extract for their antibacterial activity.

Hibiscus Rosa sinensis both leaves and petals (Plant material) [8]:

The fresh flowers and leaves of Hibiscus Rosa sinensis were collected. The flowers and the leaves washed thoroughly with tap water and then with sterile distilled water, dried in hot air oven and powdered. This powder of plant material was stored in the dry place for further using as a raw material for the extraction of antibacterial compounds.

Method of extraction by using Soxhlet apparatus:[8]

A total of 10 gm of air-dried powder of flower petals and leaves was weighed with the help of electronic weighing machine and was placed in 100 ml organic solvents, ethanol in a conical flask and then kept in a rotary shaker at 190-220 rpm for 24 hrs. And then it was filtered with the help of muslin cloth and was centrifuged at 1000rpm for 5min. The supernatant was collected and the solvent was then evaporated by solvent distillation apparatus to make the final volume of one fourth of the original volume, giving a concentration of 40mg/ml. It was stored in air tight bottles for further studies.

Testing of Antibacterial effect of leaves and petals of Hibiscus Rosa sinensis:

All *E. coli* isolates which was found to be resistant to one or more antibiotics was included in study. Lawn culture of *E. coli* isolates was done on Muller Hinton agar, resistant antibiotics alone and same with extract of leaves and petals each was placed on same Mueller Hinton Agar plate. About 15 ul containing 40 mg/ml of extract was inoculated on the plain sterile disc prepared homemade and then allowed to dry. The plates were incubated for 24hrs at 37°C.

RESULTS

During the study period, total 51 *Escherichia coli* isolates was recovered from different samples like urine, sputum, wound swab and blood which were received in the laboratory for routine testing. Identification of organism was done as per routine standard protocol and antibiotic susceptibility testing of all *E. coli* isolates was done as per CLSI guidelines[7]. In routine, *E. coli* was tested for antibiotics like ampicillin, gentamycin, nitrofurantoin, norfloxacin, ceftazidime, amikacin, cefuroxime, cefotaxime, meropenam, piperacillin, levofloxacin, ESBL panel and other antibiotics on special request.

Out of total 51 *E. coli* isolates, 35 (68.63%) *E. coli* was found to resistant to the tested antibiotics and 16 (31.37%) was found to be susceptible to all the tested antibiotics. All 35 resistant *E. coli* isolates was further tested for antibacterial effect of extract of Hibiscus Rosa sinensis both leaves and petals with the antibiotics and also the antibiotic without extract.

Out of 35 (68.37%) resistant *E. coli* isolates, 30(85.72%) were from urine, 2(5.71%) each from sputum & wound swab and 1(2.86%) from blood samples (Fig -1). Out of total 35 resistant *E. coli*, 18 (51.43%) from OPD, and 17 (48.57%) were from IPD patients. In our study, 24 (68.57%) were female patients and 11 (31.43%) male.

Out of total 35 resistant *E. coli* isolates, we found that these isolates were resistant to Ampicillin 28 (80%), ofloxacin 26 (74.28%), cefotaxime 24 (68.57%); cotrimaxazole 21 (60%); cefuroxime 21 (60%), ceftazidime 20 (57.14%), cefazolin 17 (48.57%), levofloxacin 14 (40%), piperacillin, 13 (37.14%), meropenam 11(31.43%), gentamycin 10 (28.57%) and amikacin 4 (11.43%) (Fig-2).

After testing these resistant antibiotics individually with Hibiscus Rosa sinensis leaves and petals extract, we found that few antibiotics enhances the antibacterial action more with either leaves or petals and some able to show enhancement equally with both extract (Table-1). Antibiotics like ampicillin (7.14%), ofloxacin (19.23%), cefuroxime (19.05%) and meropenem (63.63%) showed more enhancement with

petal extract than leaves. While antibiotic like cotrimaxazole (19.05%), ceftazidime (25%), cefazolin (17.65%), piperacillin (23.08%) and gentamycin (50%) showed more enhancement with leaves extract as compare to petals. But antibiotics like cefotaxime (20.83%) and amikacin (25%) showed enhancement with both extract equally. We also found that hibiscus both petal and leaves extract did not show any effect along with levofloxacin as there is no enhancement in zone size.

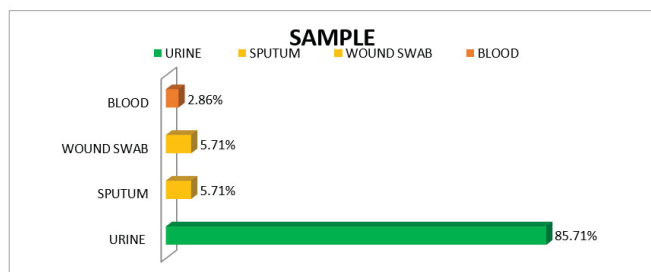


Figure 1: Sample distribution of 35 resistant isolates.

Out of 35 (68.37%) resistant *E. coli* isolates, 30(85.72%) were from urine, 2(5.71%) each from sputum & wound swab and 1(2.86%) from blood samples (Fig:1).

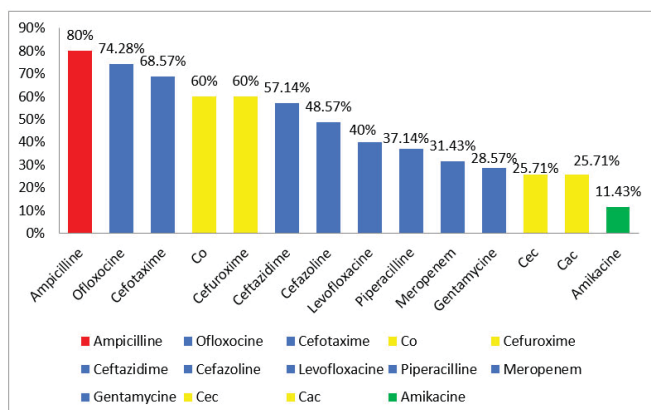


Figure 2: Distribution of resistant *E. coli* isolates as per antibiotic resistance.

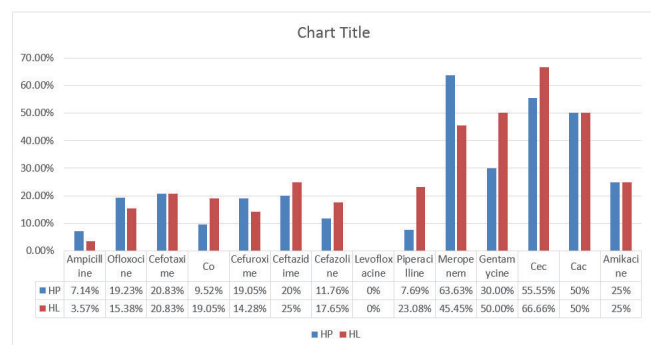


Figure 3: Combine antibacterial effect of both petal and leaves extract of Hibiscus on antibiotics.



Picture: Shows zone enhancement with extract hibiscus leaves and hibiscus petals.

DISCUSSION

Plant materials have been widely used for the treatment of infectious diseases and have gained popularity as they decrease the side effect seen with the use of systemic antimicrobials. These antibacterial properties are noticed in the active compounds that are extracted from the plants and the extraction of same depends on the solvent that is used for the extraction process. The most commonly used solvents are methanol and ethanol [9]. Considering above benefits, in the present study ethanol was used as a solvent for extraction.

The past two decades have witnessed major increase in emergence and spread of multidrug resistant bacteria and increasing resistance to newer compounds, such as fluoroquinolones and certain cephalosporins.

In our study, total 35 (68.63%) *E. coli* isolates were found to be resistant to maximum antibiotics routinely used, showing an increasing resistance trend for ciprofloxacin, trimethoprim/sulfamethoxazole and amoxicillin clavulanic acid for the *E. coli* isolates. Out of these resistant isolates, 85.71% were from urine samples.

We found that out of 35 resistant isolates, maximum resistance was shown to Ampicillin 80%, followed by ofloxacin 74.28%, cefotaxime 68.57%, 60% in both cotrimoxazole and cefuroxime. In our study, amikacin resistance was less i.e. 11.43% as compared to other and gentamycin resistance was 28.57% which was contradictory to the findings reported by Daniel *et al* that gentamycin resistance was rare in human *E. coli* isolates [4].

This antibacterial effect of the extract could be due to the presence of active compounds like flavonoids, tannins, alkaloids, triterpenoids in the extract. These active compounds may act alone or in combination to inhibit the bacterial growth. The remedies based on these plants have a minimal side effect [8,10]. Plant extract contains phenolic compounds like tannins that are very good antimicrobial agents.

We found that *Hibiscus Rosa sinensis* petal and leaves extract were equally effective against different antibiotics. The activity is attributed to their ability to complex extra cellular

and soluble proteins and with bacterial cell wall. There are several reports published on antibacterial activity of different herbal extract.

The resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plants extract are required.

CONCLUSION

The present study concludes that Hibiscus Rosa sinensis extract could inhibit human pathogen growth. The results are encouraging but precise assessment is utterly necessary before being situate in practise as well as the most active extract can be subjected to isolation of the therapeutic antimicrobials and undergo secondary pharmacological evaluation. Plant extract can be used along with antibiotics as an important supplement to enhance the antibacterial effect of antibiotics. Further, we require more studies to generate data to prove the antibacterial effect of different flower extract.

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Conflict of Interest: There is no conflict of interest.

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